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Interaction of *Salmonella telaviv* with *Maclura pomifera* Lectin

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***Salmonella telaviv*, *Salmonella tranoroa*, and *Salmonella illinois* were examined for their ability to interact with 15 purified lectins of known sugar specificity. The only interaction observed was between the lectin of *Maclura pomifera* and *S. telaviv*. *M. pomifera* lectin specifically agglutinated suspensions of *S. telaviv* and precipitated with its purified lipopolysaccharide and isolated lipid A free O polysaccharide. Quantitative inhibition assays showing methyl-α-D-galactopyranoside and *N*-acetyl-D-galactosamine to be potent inhibitors of *Maclura* lectin precipitation by *S. telaviv* O polysaccharide suggest that the interaction is mediated by D-galactose or *N*-acetyl-D-galactosamine units of bacterial polysaccharide structure, or both.**

Although lipopolysaccharides (LPSs) of groups M, 55, and E3 *Salmonella* are antigenically distinct (12), they show similarities in behavior with MOPC 384 mouse immunoglobulin A myeloma protein (13, 14). The 384 protein agglutinates group M, 55, and E3 species of *Salmonella* or sheep erythrocytes coated with group-specific LPS and precipitates purified LPS isolated from *Salmonella telaviv* (group M), *Salmonella tranoroa* (group 55), and *Salmonella illinois* (group E3). Identification of the α(1,2)-linked diglucose (kojibiose) and methyl-α-D-galactoside as specific hapten inhibitors of MOPC 384 precipitation by *S. telaviv* LPS (P. Z. Allen and J. H. Pazur, Mol. Immunol., in press) suggested the occurrence of both α-D-glucose and α-D-galactose units as structural features of LPS antigens mediating interaction with 384 myeloma protein.

In the present study, cell suspensions of *S. telaviv*, *S. tranoroa*, *S. illinois*, and their isolated LPSs were examined for their ability to interact with various lectins specific for D-glucose, D-galactose, and their corresponding 2-deoxy-2-acetamido derivatives. An interaction of isolated *S. telaviv* LPS and its lipid A free O polysaccharide, found to occur with the α-D-galactose/*N*-acetylgalactosamine [GalNAc] binding lectin of *Maclura pomifera*, was studied by quantitative precipitation and competitive inhibition.

MATERIALS AND METHODS

Polysaccharides. LPS was isolated from salmonellae (*S. telaviv*, *S. tranoroa*, and *S. illinois*) by hot phenol-water extraction and purified as described for gonococcal LPS (4). Lipid A free O polysaccharide (O-ps) was prepared from purified LPS by mild acid hydrolysis in 1% (vol/vol) glacial acetic acid at 100°C for 90 min. The hydrolysate was centrifuged at 4°C to remove insoluble lipid and extracted with chloroform and then with dimethyl ether, and the O-ps was recovered by lyophilization. Blood group A substance (Hog 1A) was isolated from hog gastric mucosa as described by Kabat (8). Guaran was provided by Irwin J. Goldstein (Department of Biological Chemistry, University of Michigan, Ann Arbor). The preparation of type 14 pneumococcal capsular polysaccharide has already been described (3).

Lectins. Affinity-purified lectins from *M. pomifera* (MPA), *Canavalia ensiformis*, *Griffonia simplicifolia* (GS I isolectin mixture and GS I B4), *Sophora japonica*, *Lens culinaris*, *Glycine max*, *Arachis hypogea*, *Ricinus communis*, *Triticum vulgaris*, and *Phaseolus limensis* were obtained from E. Y. Laboratories (San Mateo, Calif.). Preparations of purified *Dolichos biflorus* and *G. simplicifolia* isolectins (GS I A4 and GS I A3B specific for α-D-GalNAc and GS II specific for α-D-*N*-acetylglucosamine [GlcNAc]) were provided by Irwin J. Goldstein. The carbohydrate-binding specificity of these purified lectins has already been described in detail (2, 5, 6, 15, 17). A specific extinction coefficient, $E^{1\%}_{1\,cm\,(280\ nm)} = 15.7$, reported by Bausch and Poretz (2), was used to estimate the protein content of MPA solutions used for agglutination endpoint titration and quantitative precipitation.

Inhibitors. Methyl-β-D-mannopyranoside was purchased from E. Y. Laboratories. Methyl-α- and methyl-β-D-gluco- and galactopyranoside, methyl-α-D-mannopyranoside, GlcNAc, GalNAc, *N*-acetyl-D-mannosamine, ammonium 2-keto-3-deoxyoctonate, and D-glycero-D-glucoheptose were obtained from Sigma Chemical Co. (St. Louis, Mo.). 3-Acetamido-3,6-dideoxy-D-galactose was provided by Gilbert Ashwell (National Institutes of Health, Bethesda, Md.), and D-glycero-L-mannoheptose was provided by Paul A. Rebers (U.S. Department of Agriculture, Ames, Iowa). Abbreviations for sugars used in the text are D-glucopyranose (D-Glc), D-galactopyranose (D-Gal), methyl-α-D-galactopyranoside (methyl-α-D-Gal).

Agglutination. Lectin agglutination assays employing Formalin-killed suspensions of *S. telaviv*, *S. tranoroa*, and *S. illinois* were done in microtiter plates, using 50 μl of lectin solution plus 50 μl of bacterial suspension. Except for the use of Mueller-Hinton agar plates to grow salmonellae, preparation of washed, standardized bacterial suspensions and their use in microtiter endpoint agglutination assay were carried out by a standard procedure previously described in detail for gonococci (4). Lectin solutions used for agglutination were prepared in a buffer consisting of 0.1 M Tris (pH 7.3) containing 0.15 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, and 0.025% (wt/vol) sodium azide. Apart from MPA, lectins were assayed for agglutination only at 50 and 500 μg of lectin per microtiter well.

Quantitative precipitation and inhibition. Purified MPA lectin (50 μl of MPA solution with 9.0 μg of N) was mixed with different amounts of polysaccharide, and the total volume was adjusted to 0.4 ml with saline. Mixtures were incubated for 1 h at 37°C, kept at 0°C for 5 days, centrifuged, and washed twice with 0.4 ml of chilled saline. The total nitrogen content of washed precipitates was determined by the ninhydrin procedure of Schiffman (16). Sugars were assayed for their ability to inhibit precipitation of a test system consisting of 100 μg of *S. telaviv* O-ps added to 9.0 μg of MPA lectin nitrogen. The final volume of reaction mixtures was 0.4 ml. Inhibition of lectin precipitation was

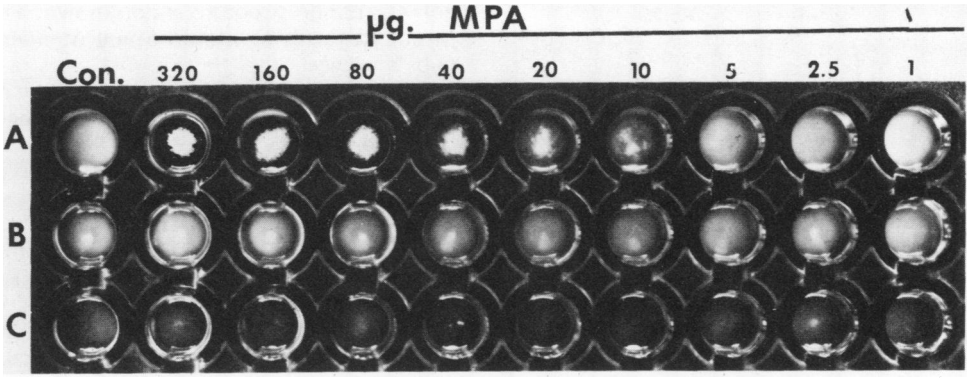

FIG. 1. Agglutination assays of *S. telaviv* (A), *S. illinois* (B), and *S. tranoroa* (C) with MPA lectin. Control wells that received no lectin are labeled Con.

assayed by the scaled-down modification of the micro Folin-Ciocalteu procedure described by Kabat and Schiffman (9). Quantitative precipitin and inhibition assays were usually carried out in duplicate, and average values were plotted. Where four or more replicate determinations were done, the arithmetic mean and the standard error of the mean were calculated and indicated by error bars in plots of data.

Agar diffusion. Gel diffusion was done in 1% agar in 0.1 M Tris buffer (pH 7.6) containing 0.15 M sodium chloride and 0.025% sodium azide, and made 1 mM with respect to $MgCl_2$, $MnCl_2$, and $CaCl_2$. Hog A substance, guaran, and type 14 pneumococcal polysaccharide were used as positive controls in diffusion against lectins.

RESULTS

Agglutination and agar diffusion. Suspensions of *S. telaviv*, *S. tranoroa*, and *S. illinois* were tested for agglutination by purified lectins (MPA, *C. ensiformis* GS I, GS I A4, GS I A3B1, GS I B4, GS II, *S. japonica*, *L. culinaris*, *G. max*, *A. hypogea*, *R. communis*, *D. biflorus*, *T. vulgaris*, and *P. limensis*). The only positive reaction obtained was the agglutination of *S. telaviv* by MPA lectin. Distinct agglutination of *S. telaviv* was obtained with 10 μg of MPA, but *S. tranoroa* and *S. illinois* were not agglutinated even by 320 μg of lectin (Fig. 1). In agar diffusion, isolated *S. tranoroa* and *S. illinois* LPSs and their corresponding O-ps's gave no band of precipitation with any of the lectins tested. However, when diffused against MPA lectin, *S. telaviv* antigens (LPS and O-ps) gave a band of precipitation that showed complete fusion with the band produced by Hog A substance.

Quantitative precipitation. Figure 2 shows the quantitative precipitation behavior of *Maclura* lectin with various *Salmonella* antigens. *S. telaviv* LPS and its lipid A free O-ps maximally precipitated 8.6 and 6.0 μg of total N, respectively, and Hog A (included as a positive control) precipitated only 4.7 μg of total N. When tested in amounts up to 280 μg, *S. tranoroa* and *S. illinois* LPS and their corresponding O-ps's failed to precipitate *Maclura* lectin. Similarly, guaran (not shown in Fig. 2) showed no precipitation when added in amounts up to 300 μg of polysaccharide.

Quantitative inhibition. Figure 3 shows the inhibition of *Maclura* lectin precipitation by *S. telaviv* O-ps that was observed when various sugars were used as competitive inhibitors. Of the sugars tested, methyl-α-D-Gal was the best inhibitor of O-ps precipitation, requiring only 8.0×10^{-2} μM for 50% inhibition. GalNAc, which required 7.6×10^{-1} μM for comparable inhibition, showed ca. one-ninth the molar inhibitory ability of methyl-α-D-Gal. Although methyl-β-D-galactopyranoside and methyl-α-D-mannopyranoside showed some inhibitory activity, they were relatively poor inhibitors of MPA precipitation: 6.8 and 8.0 μM, respectively, were required for 50% inhibition.

2-Keto-3-deoxyoctonate (11 μM), 3-acetamido-3,6-dideoxy-D-galactose (5 μM), D-glycero-D-glucoheptose (67 μM), D-glycero-L-mannoheptose (28 μM), and *N*-acetyl-D-mannosamine (1 μM) all failed to give any inhibition of O-ps–lectin precipitation.

DISCUSSION

S. telaviv, *S. tranoroa*, and *S. illinois* show similarities in their immunochemical behavior with MOPC 384 myeloma protein, mediated by portions of LPS antigen structure involving α-D-galactose and α-D-glucose residues (13, 14; Allen and Pazur, in press). Despite this similarity in behavior with monoclonal antibody, *S. telaviv* was found, in the present study, to differ distinctly from *S. tranoroa* and *S. illinois* in its behavior with the α-D-Gal/GalNAc binding lectin of MPA.

Of 15 purified lectins examined for reactivity with *S. telaviv*, *S. tranoroa*, and *S. illinois*, only a single positive interaction was observed. *S. telaviv* was readily agglutinated

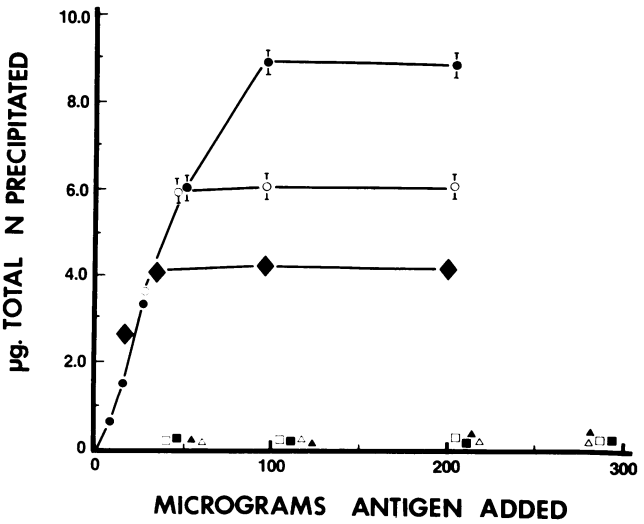

FIG. 2. Precipitation of purified MPA (50 μl with 9.0 μg of lectin N) by *S. telaviv* LPS (●), *S. telaviv* O-s saccharide (○), *S. tranoroa* LPS (■), *S. tranoroa* O-s saccharide (□), *S. illinois* LPS (▲), *S. illinois* O-s saccharide (△), Hog 1 blood group A substance (◆).

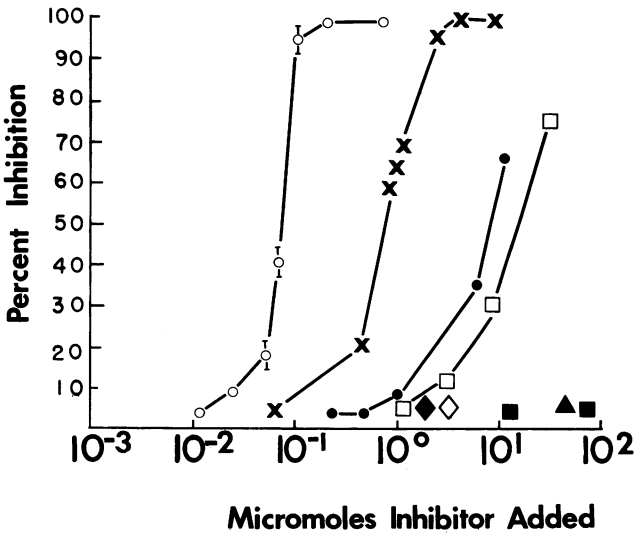

FIG. 3. Inhibition of MPA lectin precipitation (50 μl with 9.0 μg of lectin N) by 100 μg of *S. telaviv* O-ps with various sugars. Symbols indicate methyl-α-D-galactopyranoside (○), *N*-acetyl-D-galactosamine (×), methyl-β-D-galactopyranoside (●), methyl-α-D-mannopyranoside (□), methyl-β-D-mannopyranoside (■), methyl-α-D-glucopyranoside (◇), methyl-β-D-glucopyranoside (◆), GlcNAc (▲).

by MPA (Fig. 1), but not other lectins. *S. tranoroa* and *S. illinois* failed to react with *Maclura* lectin (Fig. 1) or any of the other lectins tested. That the agglutination observed is specifically mediated by LPS was shown by the ability of purified LPS and lipid A free O-ps isolated from *S. telaviv* to precipitate MPA although the corresponding antigens of *S. tranoroa* and *S. illinois* showed no reactivity (Fig. 2). Purified *S. telaviv* LPS precipitates 95% of the lectin N used in precipitation assays; its corresponding O-ps removes only 67% of the lectin added. Differences in the maximum amount of *Maclura* lectin precipitated have been previously reported for various blood group antigens (15). Whether the difference in lectin N precipitated by *Salmonella* polysaccharides is due to a decreased solubility or to dissociation of LPS-lectin complex is not known and may be associated with the presence of a hydrophobic lipid A moiety in the LPS ligand.

The binding site specificity of MPA has been examined in detail by inhibition of lectin interaction with blood group antigens (2, 15) and α-glycosides of D-Gal and D-GalNAc found to be the most potent inhibitors of precipitation (15). Of the sugars assayed (Fig. 3), methyl-α-D-Gal and GalNAc were found to be the best inhibitors of *S. telaviv* O-ps–MPA interaction. The relative ability of sugars found in the present study to inhibit lectin precipitation by *S. telaviv* O-ps (methyl-α-D-Gal > D-GalNAc) is in agreement with comparable inhibition data obtained for blood group antigens (15).

D-Glc, D-Gal, D-GalNAc, 3-amino-3,6-dideoxyhexose, heptose, and 2-keto-3-deoxyoctonate have been identified as components of both *S. telaviv* and *S. tranoroa* LPS (11). D-GalNAc occurs as a constituent of O-specific polysaccharide, although repeating unit structures for O-specific chains of these LPSs have not yet been elucidated (11, 12). The complete outer core structure common to all smooth *Salmonella* LPSs consists of a pentasaccharide composed of α-D-Glc, α-D-Gal, and α-D-GlcNAc in molar ratios of 2:2:1 (10, 12). Whether D-Gal units of *S. telaviv* and *S. tranoroa* LPS are confined only to the common outer core region or occur as components of both common core and O-specific polysaccharide structure is not known. The failure of MPA to interact with *S. tranoroa* and *S. illinois* LPSs or their O-ps's suggests that the α-D-Gal units of *Salmonella* common core structure are not generally accessible for lectin interaction. This finding is not surprising since the stereochemical environment in which specific sugars are located has been shown to influence the reactivity of *Salmonella* LPS with lectins (1, 7).

The occurrence of D-Gal and D-GalNAc as constituents of *S. telaviv* LPS (11), together with inhibition data shown in Fig. 3, indicate that interaction with MPA is mediated by D-Gal or D-GalNAc residues, or both, of *S. telaviv* polysaccharide. Whether lectin-reactive sugars are located entirely in the O-specific chain or include the common outer core region of *S. telaviv*, polysaccharide structure cannot be established by present data. A structural basis for the failure of α-D-Gal and α-D-GalNAc binding lectins (*S. japonica*, *D. biflorus*, GS I A4, and GS I B4) to precipitate MPA-reactive *S. telaviv* O-ps is not clear. This pattern of ligand behavior may reflect differences in the fine specificity among lectins (15) and the relative position and stereochemical environment in which D-Gal and D-GalNAc residues are located (1, 7).

LITERATURE CITED

1. **Ahmed, N. M., J. Radziejewska-Lebrecht, C. Wideman, and H. Mayer.** 1980. Reactivity of isolated lipopolysaccharides of enterobacterial R mutants with complete and incomplete core structures with lectins. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **247**:468–482.
2. **Bausch, J. N., and R. D. Poretz.** 1977. Purification and properties of the hemagglutinin from *Maclura pomifera* seeds. Biochemistry **16**:5790–5794.
3. **Connelly, M. C., and P. Z. Allen.** 1983. Chemical and immunochemical studies on lipopolysaccharides from pyocin 103-sensitive and resistant *Neisseria gonorrhoeae*. Carbohydr. Res. **120**:171–186.
4. **Connelly, M. C., D. C. Stein, F. E. Young, S. A. Morse, and P. Z. Allen.** 1981. Interaction with lectins and differential wheat germ agglutinin binding of pyocin 103-sensitive and -resistant *Neisseria gonorrhoeae*. J. Bacteriol. **148**:796–803.
5. **Goldstein, I. J., D. A. Blake, S. Ebisu, T. J. Williams, and L. A. Murphy.** 1981. Carbohydrate binding studies on the *Bandeiraea simplicifolia* I isolectins. J. Biol. Chem. **256**:3890–3893.
6. **Goldstein, I. J., L. A. Murphy, and S. Ebisu.** 1977. Lectins as carbohydrate binding proteins. Pure Appl. Chem. **49**:1095–1103.
7. **Goldstein, I. J., and A. M. Staub.** 1970. Interaction of concanavalin A with polysaccharides of *Salmonella*. Immunochemistry **7**:315–319.
8. **Kabat, E. A.** 1956. Blood group substances; their chemistry and immunochemistry, p. 135–139. Academic Press, Inc., New York.
9. **Kabat, E. A., and G. J. Schiffman.** 1962. Immunochemical studies on blood groups XXVIII: further studies on the oligosaccharide determinants of blood group B and BP1 and specificity. J. Immunol. **88**:782–789.
10. **Luderitz, O., M. A. Freudenberg, C. Galanos, V. Lehmann, E. Reitschel, and D. H. Shaw.** 1982. Lipopolysaccharides of gram-negative bacteria. Curr. Top. Membr. Transp. **17**:79–151.
11. **Luderitz, O., E. Ruschmann, O. Westphal, R. Raff, and R. Wheat.** 1967. Occurrence of 3-amino-3,6-dideoxyhexoses in *Salmonella* and related bacteria. J. Bacteriol. **93**:1681–1687.
12. **Luderitz, O., O. Westphal, A. M. Staub, and H. Nikaido.** 1971. Isolation and chemical and immunochemical characterization of bacterial lipopolysaccharides, p. 145–233. *In* G. Weinbaum, S. Kadis, and S. J. Ajl (ed.), Bacterial toxins, vol. 4. Academic Press, Inc., New York.
13. **Potter, M.** 1970. Mouse IgA myeloma proteins that bind polysaccharide antigens of enterobacterial origin. Fed. Proc.

29:85–91.
14. **Potter, M.** 1971. Antigen-binding myeloma proteins in mice. Ann. N.Y. Acad. Sci. **190**:306–321.
15. **Sarkar, M., A. M. Wu, and E. A. Kabat.** 1981. Immunochemical studies on the carbohydrate specificity of *Maclura pomifera* lectin. Arch. Biochem. Biophys. **209**:204–218.
16. **Schiffman, G.** 1966. Immunological methods in characterizing polysaccharides. Methods Enzymol. **8**:79–85.
17. **Wood, C., E. A. Kabat, S. Ebisu, and I. J. Goldstein.** 1978. An immunochemical study of the combining sites of the second lectin isolated from *Bandeiraea simplicifolia* (BS II). Ann. Immunol. (Paris) **129**:143–158.